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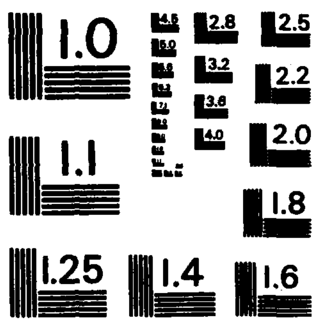
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EFFECTS ON THE GROWTH AND SURVIVAL OF EGGS AND EMBRYOS OF THE CALIFORNIA GRUNION (*LEURESTHES TENUIS*) EXPOSED TO TRACE LEVELS OF TRIBUTYLTIN

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This experiment was conducted to determine the effects of various dose levels of tributyltin (TBT) on the eggs, embryos, and larvae of the California grunion (Leuresthes tenuis). Both pre- and postfertilization effects were examined. The study demonstrated that the presence, during embryonic development, of tributyltin cation derived from antifouling coating leachates at concentrations from 0.14 to 1.72 ~~µg~~ TBT/L had no adverse effects on either the hatch success or growth of embryonic fish. Continuous exposure to water concentrations of TBT at and below 1.72 µg/L had no adverse effects on embryonic development and, in fact, significantly enhanced hatch success and stimulated growth suggesting a hormestic effect. Exposure of hatched larvae to similar concentrations of TBT for 7 days did not significantly affect survival. High dosing of TBT (74 µg/L) during fertilization, representing possible chemically available interstitial concentrations, reduced hatching success by about 50 percent over controls. Similar dosing at 10 µg/L showed no significant reduction in hatching success.

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SUMMARY

This experiment was conducted to determine the effects of various dose levels of tributyltin (TBT) on the eggs, embryos, and larvae of the California grunion *(Leuresthes tenuis)*. Both pre- and postfertilization effects were examined. The study demonstrated that the presence, during embryonic development, of tributyltin cation derived from antifouling coating leachates at concentrations from 0.14 to 1.72 µg TBT/L had no adverse effects on either the hatch success or growth of embryonic fish. Continuous exposure to water concentrations of TBT at and below 1.72 µg/L had no adverse effects on embryonic development and, in fact, significantly enhanced hatch success and stimulated growth suggesting a hormestic effect. Exposure of hatched larvae to similar concentrations of TBT for 7 days did not significantly affect survival. High dosing of TBT (74 µg/L) during fertilization, representing possible chemically available interstitial concentrations, reduced hatching success by about 50 percent over controls. Similar dosing at 10 µg/L showed no significant reduction in hatching success.

BACKGROUND

The U.S. Navy has made the decision to implement tributyltin containing antifouling coatings into the Fleet based on interim environmental documentation that has been completed and approved (Federal Register Vol. 50, No. 120, 21 Jun 85) in accordance with the National Environmental Policy Act. This decision is based on a critical need to substantially reduce the amount of propulsion fuel used by the Fleet and the frequency of overhaul periods and to increase operational capabilities.

To evaluate the environmental ramifications of the Fleet use of organotin containing coatings, from both drydock discharges and the leaching of tributyltin from hulls, information on the effects of tributyltin leachates on marine organisms is needed. Marine fishes represent a significant segment of the ecosystem both from their importance in commercial and recreational fishing and their prominence in the food chain, including human consumption. Generally, the success of reproduction, represented by fertilization, embryo development, hatching, and larval survival, is a sensitive indicator of the potential impact of a pollutant on fish populations. This study used the California grunion to investigate the effects of organotin on fish reproductive success.

INTRODUCTION

The introduction of very small quantities of hazardous substances into the marine environment may have important ecological effects, creating regulatory problems for the dischargers responsible for the toxicants. Such subtle introductions can range from partially used pesticides dumped into residential drains to the slow leaching of biocides from antifouling paints into small marine boat basins. Tributyltin (TBT) compounds are the major active components of controlled release antifoulant paints proposed for use on Navy vessels. Organotin-based paints are in commercial use on pleasure and commercial craft and are being tested for power plant cooling intake structures. An increase in TBT input may be expected in some harbors used by the Navy. U'Ren (1983) has indicated that ultratrace TBT doses (<1 μg/L = ppb) are acutely toxic to at least one species of marine copepod, and these levels may already be present in some low circulation yacht harbors. Because of environmental concerns and the requirement for an environmental assessment, the Naval Ocean Systems Center (NOSC) undertook an investigation to examine the toxic effects of TBT on nontarget marine species.

In a recent review of the organotin literature (KLI, 1984), a qualitative scheme was developed to rank past testing. Three principal parameters were used:

1. Type of bioassay method (e.g., static, static renewal, or flowthrough).

2. Reporting of actual organotin measurements.

3. Quantification and identification of actual toxicant species in the test solution (e.g., monobutyltin, dibutyltin, or tributyltin).

Kinetics Laboratories, Inc. suggested the physical nature of the bioassay performed may significantly affect chemical parameters, control of biological processes, and interpretation of test results. Flowthrough bioassay methods were generally considered the most reliable for maintaining a healthy environment for test species in regard to waste removal, oxygenation, pH, temperature control, as well as a steady introduction of toxicant. Static renewal bioassay testing was considered a second alternative, while static bioassay testing was ranked third. A score of three was assigned to studies where flowthrough bioassay testing methods were used. Static renewal testing studies were given a score of two, and static testing studies received a score of one. Studies where measurements of the organotin were made by accurate methods capable of ultratrace speciation and detection received a score of two. Studies where nonspecific methods of measuring the organotin toxicant in solution were used received a score of one. A score of zero was assigned to studies where nominal toxicant concentrations were reported based on calculated estimates, such as weight to volume concentrations. A maximum score of five (three for flowthrough bioassay testing, plus two for ultratrace detection and speciation of organotin toxicant) was thus possible for studies where the most reliable bioassay testing procedure and analytical support methods were employed.

The review revealed while quite a bit of toxicological testing has been conducted on marine and freshwater organisms (44 species tested), only 7 species studied received a rating of four and none were assigned a rating of five. Additionally, of the 16 species of fish tested, only two tests

(*Solea solea* -- Thain, 1983; and *Cyprinodon variegatus* -- Ward et al., 1981) were conducted on early life history stages. Niimi (1983) has pointed out that the management of a renewable resource, such as fish, is based upon the premise that a species will perpetuate itself, but information such as that for TBT indicates the presence of trace levels may degrade environmental quality and result in an overall reduction in survival rates.

The expression of various types of metabolic and behavioral responses to toxic substances is not solely dependent upon the structure of an individual's genetic material; it is also a consequence of the environment within which a developing organism subsists. The quality of environmental factors present during the development of an organism from fertilization to adult determines where, within the range of genotypic possibilities, a single phenotype will prevail. Selection acts upon the individual, and the success of the population is intimately associated with the survival of the individual (Dobzhansky et al., 1977). Additionally, large, robust individuals that develop more rapidly than their cohorts are commonly believed to be reproductively more successful. Thus, environmental conditions, restrictive to rapid growth and development of an individual, may have profound effects on the ultimate survival and success of the population year class. Rosenthal and Alderdice (1976) have suggested that early embryos and prolarvae are often the most sensitive to toxicants, and Lasker (1982) has indicated that year class strength is dependent upon the survival of larval and juvenile fish.

Because of the lack of information on the toxic effects of TBT on the critical and sensitive early life history stages of fish, this study was conducted to determine the tolerance of fish gamates and fertilized eggs exposed to various levels of TBT. By taking advantage of a sophisticated dosing system and a state-of-the-art organotin speciation technique developed at NOSC, the experiment was designed to receive a rating of five on the previously mentioned scale. The choice of a test species was based on three criteria: (1) eggs must be deposited in the sediment, where exposure to TBT would be greatest; (2) the larvae had to be amenable to laboratory culture; and (3) a large number of eggs had to be available. The California grunion, *Leuresthes tenuis*, adequately satisfied the above parameters. Grunion eggs are deposited in sandy sediments where prelarval development occurs and, while not an obligate bay species, grunion have been known to spawn in San Diego Bay. Early life stages are easily cultured in the laboratory from eggs (Ehrlich & Farris, 1971; May 1971), and eggs are obtainable biweekly during the spawning season, which extends from March through August (Walker, 1952).

Additionally, grunion have attracted the attention of numerous researchers who have studied lactic acid responses (Scholander et al., 1962); light effects (McHugh, 1954; Reynolds et al., 1977); salinity tolerance (Reynolds et al., 1976); egg size (Moffatt & Thomson 1978); feeding behavior (May 1971); parasitism (Olson, 1972, 1977); Canning & Olson, 1980); p,p-DDT sensitivity (Valentine & Soule, 1973); benzo(a)pyrene sensitivity (Puffer et al., 1979); and cadmium sensitivity (Thum & Newton, 1980). The taxonomy of the California grunion is described by Moffatt and Thomson (1975), and a taxonomic key is provided by Miller and Lea (1972). Embryonic and early life stages have been described by David (1939).

This study addressed the following null hypotheses (H_0):

1. H_0 = postfertilization exposure of grunion eggs to low doses of TBT has no effect on hatch success or notochord length at time of hatch.

2. H_0 = exposure of grunion eggs and sperm during fertilization and subsequent low-level dose incubation has no effect on fertilization, hatch success, or notochord length at the time of hatch.

METHODS

Ripe adult grunion were collected during the first night of their bimonthly spawning run on Ocean Beach (San Diego) from 2230 to 2345 hours, 1 June 1984. Eggs from live, spawning females were directly stripped into three 250-ml polycarbonate canisters, which contained 25 ml of 0, 10, or 74 μg/L TBT and filtered seawater. Field dosing (10 and 74 μg/L) prior to fertilization was performed to simulate interstitial water concentrations. After one female was stripped into each of the three containers, milt from three males was sequentially added, permitting dose-specific fertilization to occur. This process was repeated until 45 females and 45 males (15 per first dose level) had been acquired. Continuous/sequential fertilization was necessary because the chorion hardens with time, independent of fertilization, and drastically reduces "fertilizability" (Thum & Ehrlich, 1979). At the end of fertilization, the mixture was poured through a dose-specific 0.5-mm mesh Nytex screen and thoroughly rinsed with appropriately dosed seawater. This was done to reduce fungal problems previously encountered (Clark, 1925).

Clark (1925) has shown that while the number of mature ova varies with length and age of the female, an average of 2,000 mature ova per female can be expected. Thus, the number of potential ova from 15 females was assumed to be sufficient to provide the number of eggs required for this experiment and assure an adequate genetic representation of the local population.

In the field, approximately 30,000 fertilized eggs were transferred to each of three new polycarbonate containers holding 25 ml of the appropriate dose level of TBT. The closed containers were placed in a 20-L bucket of ambient temperature seawater (19.5 °C) and transported to the NOSC Marine Science Laboratory test facility at Point Loma, California.

Upon arrival at the laboratory, randomly selected eggs from each field dose level were mixed with coarse silica sand (1 to 2 mm) at a ratio of approximately 1,000 eggs to 150 ml of sand. This mixture was placed in an incubation apparatus modeled after that of Ehrlich and Farris (1971). In all, 30 incubators were made and distributed into the dosing system according to Table 1. The entire test system required 30,000 eggs, and the unneeded eggs were discarded.

Table 1. Distribution of replicate incubators (three per dose combination) within dose combination levels. A dash indicates no testing at the dose combination.

Field Dose (ppb)	Mean Laboratory Dose (μg/liter TBT) 0	0.05 ±0.006	0.14 ±0.075	0.33 ±0.057	0.90 ±0.323	1.72 ±0.652
0	3	3	3	3	3	3
10	3	-	-	-	-	3
74	3	-	-	-	-	3

The dosing system used had been in operation for over 2 months prior to the start of the experiment and is described in detail elsewhere (Valkirs, Davidson, and Seligman, in press). Briefly, 1-square-foot polycarbonate plates were painted with SPC-954 antifouling paint (International Paints, Inc.). This paint is a self-polishing copolymer formulation where tributyltin is chemically bound to the paint matrix. The paint composition is listed by the manufacturer as 9.4-percent tributyltin methacrylate bound as a polymer, 0.5-percent bis (tributyltin) oxide, 44.7-percent cuprous oxide, and 45.5-percent inert ingredients. To obtain a stable dose concentration, the plates were "aged" by soaking in rapidly flowing seawater for 30 days and then mounted in one of six polycarbonate leaching troughs. Polycarbonate was used because of its low adsorptivity to organotins (Dooley & Homer, 1983). Dose levels were adjusted by increasing the number of painted plates exposed to flowing seawater.

In addition to the continuous release of tributyltin, a minor amount of copper was also released into the seawater treatments. Seawater samples were collected and measured for copper by graphite furnace atomic absorption detection. Only the highest treatment (1.72-ppb TBT) reached copper at a concentration above that measured in ambient incoming seawater. The amount of copper introduced into the 1.72-ppb TBT treatment was measured as 2.5-ppb total copper. This concentration was less than twice the tributyltin concentration measured in the treatment seawater (1.72-ppb) and, therefore, was not considered toxic relative to the amount of tributyltin present. In a recent study, U'Ren (1983) reported tributyltin was at least 10 times more toxic to copepods than copper. A tributyltin-to-copper ratio of 0.69 in the only test solution where copper was measurable above background levels suggested that copper probably did not contribute to the observed toxic effects. The distribution of toxicant was made possible by a multiport header tank and PFE Teflon tubing. Flow rates (180 ml/min) to individual incubation chambers were adjusted and maintained via a fixed glass orifice at the discharge end of the Teflon tubing. After 10 days of incubation at an average water temperature of 20.5 °C, or 4,920 °C-hours, the larvae were ready to hatch.

Hatching was initiated by depositing eggs and sand from an incubation apparatus into a 3-L glass crystallizing dish containing 2-L of 18 °C seawater. The mixture was gently swirled with a plastic spatula. The eggs, suspended in the water column, settled to the center of the container above the sand, and within 2 minutes began to hatch. A hatch time of 15 minutes was permitted to elapse before the introduction of the anaesthetizing substance, tricaine methanesulfonate (MS222), and within 2 minutes all swimming motion

and hatching had ceased. Before the application of MS222 to the 0.0 ppb-lab/0.0 ppb-field treatments, 180 larvae were removed for an additional 7-day TBT sensitivity study. The results of this larval study are presented in Appendix A.

Unhatched eggs were removed, dipped twice in distilled water, divided into two groups (fertilized/unhatched and unfertilized/opaque), enumerated, and placed in clean glass containers for storage. All larvae were removed, counted, rinsed in distilled water, and placed in clean glass vials for storage. Ten randomly selected larvae from each replicate were also measured for notochord length and examined for anomalous development. All sample vials were then archived and frozen for future tissue tin analysis.

As this experiment was conducted simultaneously with several long-term chronic TBT studies, physical and chemical water parameters were measured in conjunction with these other studies. Measurements of dissolved oxygen and temperature were made daily, and the TBT concentration was measured weekly. Measurements and speciation of the organotin compounds in the dose water were made by a modified hydride derivatization technique adapted from Hodge et al. (1979). Tin detection was accomplished by hydrogen-air flame atomic adsorption spectrometry detection following purge and trap collection of volatile hydrides. The speciation of organotins was accomplished by separation on the basis of differential hydride derivative boiling points as they evolved from a cryotrap. Braman and Tompkins (1979) have used this method to measure inorganic and methyltin compounds in environmental samples at subnanogram per liter (parts per trillion (ppt)) concentrations.

Data analyses were of two types: descriptive statistics and hypothesis testing. The descriptive statistics used were the arithmetic mean and standard error. Since the initial distribution of fertilized eggs was random and qualitative, all incubation containers contained different numbers of eggs. It was, therefore, not appropriate to compare absolute hatch numbers between replicates or treatments. Thus, calculations are presented as a percentage of the total number of eggs within a replicate, and this total is considered to be equal to the number of larvae, plus the number of fertilized/unhatched eggs, plus the number of unfertilized/opaque eggs.

The types of analyses used to test the null hypothesis were one-way and two-way analysis of variance (ANOVA) (Snedecor & Cochran, 1978). The Student-Newman-Keuls (SNK) multiple range test (Sokal & Rohlf, 1969; Snedecor & Cochran, 1978; Steel & Torrie, 1980), an *a posteriori* test, was also used. Where appropriate, ANOVAs were run to assess the relationships of between replicate and within replicate variability. When between replicate (container) variability was much smaller than within container (individual) variability, individuals were considered to be replicates. This procedure vastly increases the degrees of freedom in the error term and concomitantly the power of the test.

Two-way ANOVAs present the possibility of an interaction between treatments. A significant second-order interaction (e.g., field dose times lab dose) in the ANOVA invalidates the F test for the main effects (Clarke, 1980;

Doherty, 1983). When this occurred, treatments were combined (e.g., field dose-lab dose) and a one-way ANOVA was performed. The only information taken from this analysis was the sums of squares permitting the computer software to perform an SNK ranking (α=0.05).

Error inductive parameters within an experiment are often uncontrollable or unknown and may leave some initial assumptions unsatisfied. The inability to precisely fulfill all assumptions of a specific test does not preclude the use of that test. Hypothesis tests are tools of inference and deviations from assumptions, for instance lack of normality, do not necessarily negate the use of a particular test (e.g., ANOVA). However, the alpha (α) levels used to set limits of confidence may well be distorted. This may lead to an increased probability of committing Type I or II errors; that is, a rejection of a true null H_0 or acceptance of a false null H_0 (Sokal & Rohlf, 1969). For these reasons rigid hypothesis testing, such as establishing a rejection of H_0 at $\alpha<0.05$ and accepting all results at $\alpha>0.05$, was not conducted. Instead a range of acceptance values was used: $\alpha>0.1$ -- not significant; $0.01<\alpha<0.10$ -- marginally significant; and $\alpha<0.01$ -- very significant. Alpha levels used in this study were calculated directly and not taken from tables.

RESULTS

Figure 1 depicts the actual tracking of dose concentrations for 2 months. During the grunion experiment there was good separation between dose levels, and the doses were close to target values of 0.00, 0.05, 0.20, 0.30, 0.80, and 2.0 ppb. The periodic, brief spikes in concentration are assumed to be the result of a bacterial and/or diatom layer on the TBT painted panels sloughing off. Periodic sloughing would presumably re-expose "biologically bound" areas, permitting a higher leach rate, which would in turn be followed by a slow re-accumulation of the biological film.

Since the experiment addresses two distinctly different null hypotheses, the results pertinent to each will be presented separately.

1. **The effect of postfertilization dose of TBT on hatch success and notochord length.** Table 2 is a summary of the postfertilization dose study. Note the number of potential eggs used within each replicate was not equal due to the initial random distribution of eggs to the incubation chambers. Hatch success comparisons have been made based upon percentages of the total number of eggs within a replicate. In all, 6,465 eggs and larvae were examined and enumerated. The 0.05-ppb dose information is not included in the analyses because of a 24-hour stoppage in water flow to this treatment. The "no-flow" situation near the end of prolarval development killed all but 14 of the 1,046 eggs at this level and made use of this treatment unacceptable.

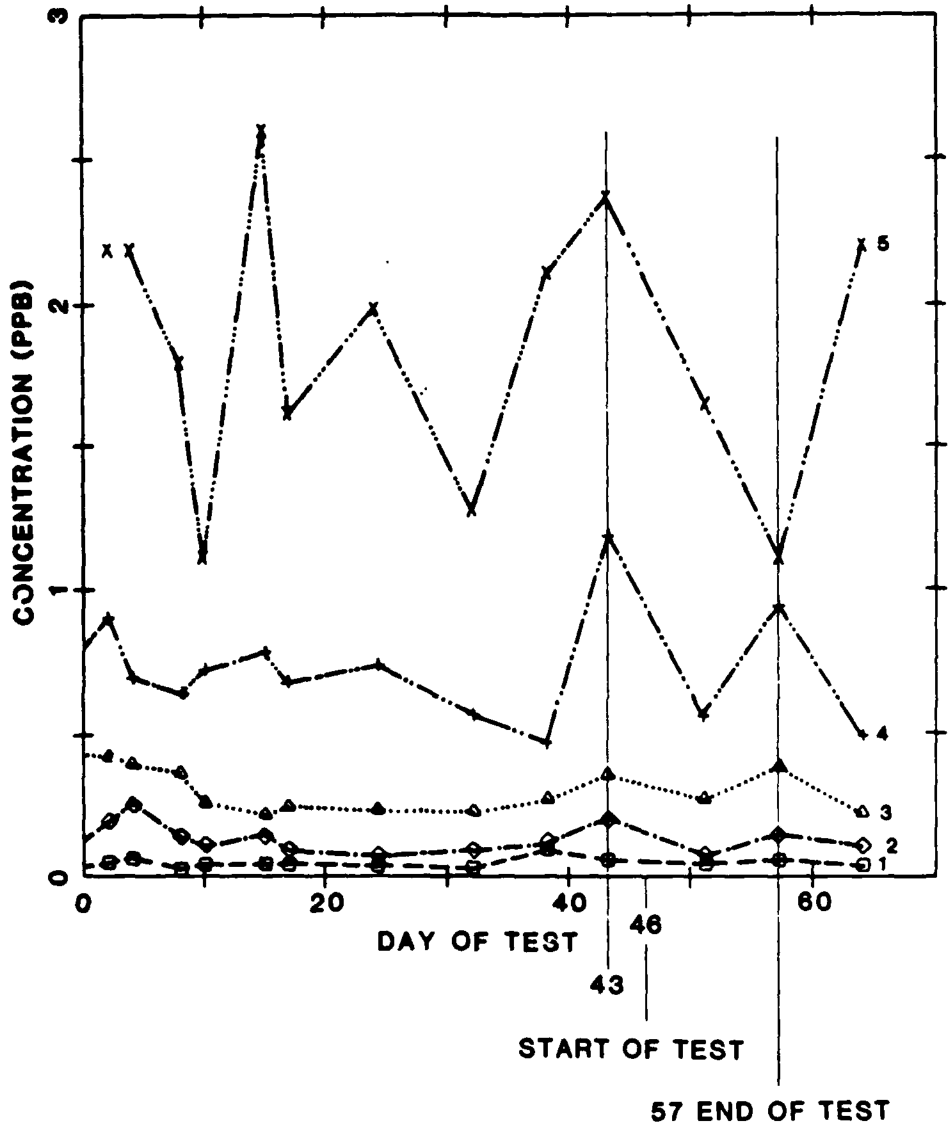

Figure 1. Actual measured TBT concentrations for the 2-month testing period. The grunion egg test took place from day 46 to day 57.

Table 2. Summary of test results from laboratory-dosed grunion eggs.

Target Dose Level (ppb)	Actual Dose Level (ppb)	Mean Notochord Length (mm)	n	Mean Percent Hatched	n
0.0	0.0	7.02 (SE = 0.11)	10	67.44 (SE = 2.95)	3
0.20	0.14 ± 0.075	7.33 (SE = 0.07)	10	68.87 (SE = 0.27)	3
0.30	0.33 ± 0.057	7.45 (SE = 0.06)	10	69.77 (SE = 7.77)	3
0.80	0.90 ± 0.323	7.46 (SE = 0.06)	10	75.16 (SE = 2.10)	3
2.00	1.72 ± 0.652	7.41 (SE = 0.08)	10	80.77 (SE = 2.05)	3

Hatch success, represented by percent hatch, was not significantly affected by laboratory dose levels (α=0.1780) and ranged from 67.44 percent - standard error (SE) = 2.95 (0.0 ppb) to 80.77 percent - SE = 2.05 (1.72 ppb). Notochord length at hatch, ranging from 7.02 mm - SE = 0.11 (0 ppb) to 7.46 mm -- SE = 0.06 (0.90 ppb), was marginally significantly affected by dose level (α=0.0685) although the SNK could not distinguish between treatment levels.

2. **The effects of three prefertilization field doses and two continuous postfertilization laboratory doses of TBT on fertilization, hatch success, and notochord length at hatch.** This portion of the investigation, summarized in Table 3, examined the effects when TBT is present during the fertilization process and the possibility of an interaction between pre- and postfertilization dosing. The percentage of unfertilized eggs, ranging from 6.33 percent - SE 0.98 (74 ppb field/1.72 ppb lab) to 8.68 percent - SE 0.68 (0.0 ppb field/1.72 ppb lab), was not significantly affected by TBT (α=0.1311), and no interaction was evident between field and laboratory dosing.

Table 3. Summary of test results from field and laboratory-dosed grunion eggs.

Field Dose Level (ppb)	Lab Dose Level (ppb)	Mean Notochord Length (mm)	n	Mean Percent Hatched	n	Mean Percent Unfertilized	n
0.0	0.00	7.02 (SE=0.11)	10	67.44 (SE=2.95)	3	8.30 (SE=1.11)	3
0.0	1.72	7.41 (SE=0.08)	10	80.77 (SE=2.05)	3	8.68 (SE=0.68)	3
10.0	0.00	7.23 (SE=0.05)	10	58.85 (SE=4.63)	3	6.75 (SE=1.70)	3
10.0	1.72	7.32 (SE=0.05)	10	67.61 (SE=4.04)	3	6.59 (SE=0.83)	3
74.0	0.00	7.13 (SE=0.06)	10	33.42 (SE=3.57)	3	6.47 (SE=0.56)	3
74.0	1.72	7.24 (SE=0.04)	10	68.67 (SE=3.46)	3	6.33 (SE=0.98)	3

A two-way ANOVA performed on notochord length and percent hatch success indicated very significant interactions between field and laboratory dosing (α=0.0005 and α=0.0056, respectively). Combining the main effects, field and laboratory doses (ppb field - ppb laboratory), and re-analyzing the SNK multirange test produced the results in Tables 4 and 5, at an alpha level of 0.05. Thus, with respect to hatch success, there appears a trend where laboratory-dosed eggs experience greater hatch success than their undosed counterparts. Three distinct SNK groupings are evident with the 0.00 - 1.72 ppb (80.77 percent) at one extreme and the 74-0.0 ppb (33.42 percent) at the other. All other dose combinations, ranging from 58.85 to 68.62 percent

success, group together. Examination of Table 4 also indicates that within a laboratory dose grouping (0.00 or 1.72 ppb) the hatch success of untreated field eggs was generally significantly greater than TBT treated eggs. In fact, hatch success of the field controls (0.0 - 0.0 ppb) was over twice that of the high field dose (74 - 0.0 ppb).

Table 4. SNK ranking (α=0.05) of percent hatch results. Due to a significant interaction the main effects have been combined. Means with the same grouping letter are not significantly different.

Grouping	Mean	n	Interact	
A	80.767	3	0 ppb	1.72 ppb
B	68.617	3	74 ppb	1.72 ppb
B				
B	67.612	3	10 ppb	1.72 ppb
B				
B	67.444	3	0 ppb	0 ppb
B				
B	58.852	3	10 ppb	0 ppb
C	33.423	3	74 ppb	0 ppb

Table 5. SNK ranking (α=0.05) of notochord length at hatch. Due to a significant interaction the main effects have been combined. Means with the same grouping letter are not significantly different.

Grouping	Mean	n	Interact	
A	7.4106	30	0 ppb	1.72 ppb
A				
B A	7.3168	30	10 ppb	1.72 ppb
B				
B C	7.2363	30	74 ppb	1.72 ppb
B C				
B C	7.2256	30	10 ppb	0 ppb
C				
C	7.1264	30	74 ppb	0 ppb
D	7.0245	30	0 ppb	0 ppb

Notochord length follows a similar pattern, with laboratory-dosed groups being more successful (larger) than their counterparts. SNK analysis overlapped the A and C groupings with the B grouping; thus, only two groups are significantly different at the 0.05 level: group D (0.0 - 0.0 ppb) and groups A, B, and C combined. There additionally appears to be a similar trend of field dose adversely affecting length, as was the case for hatch success but only with respect to the 1.72-ppb laboratory treatment. The opposite case occurs in the 0.0-ppb laboratory dose, where the control (0.0 - 0.0 ppb) larvae were significantly smaller than the treatment larvae.

DISCUSSION

While not comfortably distinct, the results of this study do point to an interesting and not uncommon problem in toxic substances testing -- some treatments appeared to fare better than controls. When considering laboratory dosing only, the treatment group exposed to the greatest concentrations of TBT had a 16.6-percent hatch advantage over the control group. Notochord length at hatch was also the smallest in the control groups for this test scenario. The field-dosed fertilization portion of this study indicated similar trends, as evidenced by the separation of the larger and more successful 1.72-ppb dose groups from the 0.0-ppb groups. Within laboratory treatments, however, field dosing appeared to reduce hatch success in both the dosing protocols and reduced notochord length in the 1.72-ppb laboratory group. In the 0.0-ppb laboratory-dosed group, TBT significantly stimulated the mean growth of larvae above the controls. Thus, it appears that TBT is both detrimental and beneficial to the California grunion. This is possibly the result of testing at toxicant levels near the lower threshold of what might be classed as a "no effect" dose. At this limit, very small changes in laboratory parameters (e.g., lighting, flow rate, and temperature), if differentially applied to treatments, can radically affect experimental results. Additionally, threshold levels are based on an examination of a "mean" population response, and the impact of individual genotypic variability induces unpartitionable errors into the statistical analysis process. These inestimable errors may help to obscure test results. Stebbing (1982) with coelentrates, Laughlin et al. (1983) with mud crab zoea, and, to a lesser extent, Seinen et al. (1981) with trout have indicated similar results when working with low dose levels of organotins. In these investigations some degree of negative toxic response and positive stimulation were noted. Hormesis is the term applied when such stimulatory effects are caused by low levels of a potentially toxic substance (Stebbing, 1982) and is often overlooked, ignored, or explained away by "high replicate variability."

Historically, the phenomenon has become evident when dose levels have been reduced to determine threshold level effects of various toxicants. Arndt-Schulz (1887) originated the idea when he found that many toxic chemicals stimulated the growth of yeast, and, later, Hueppe (1896) confirmed the results with bacteria. For 45 years, Arndt-Schulz Law of Hueppe's Rule was used to describe the phenomenon and support one of the principles of homopathic medicine (which held that the power of drugs increased with their dilution). Southam and Ehrlich (1943) coined the word "hormesis" to describe a stimulatory effect caused by subinhibitory concentrations of any toxic substance on any organism.

Hormesis has recently become more apparent (although seldom reported as such) as toxicity testing strategies have tended toward "environmental levels." This has generally been brought about by a coupling of two very different events: a major increase in the ability to measure trace quantities of pollutants in the environment and an elevated "environmental consciousness." These two driving forces have combined to push aquatic toxicology to its current scientific and technological limits. Work conducted by U'Ren (1983) showed at least some organisms are surviving in environments where levels of contaminants are at or above the lethal toxic limits predicted by current single species testing methods. Possibly, the entire philosophy of single species testing at environmental levels is inadequate. In our striving to "physically" become more "environmentally realistic," we have generally ignored a basic "biological" reality -- no one species exists independently of other species. Interactions, as evidenced by thousands of ecological studies, do take place between individuals, interspecific groups, and the environment; to believe that at ultralow dose levels single species testing will provide resource managers with any more useful information than high dose level studies have provided in the past is extremely naive. Thus, it appears the time has come to re-think present toxic testing strategies and redirect our energies toward a more general "biological" reality, that of multispecies testing.

In summary, this study indicates that the presence, during embryonic development, of low levels of TBT appears to have no adverse effects (growth or hatch success) on California grunion. Continuous exposure to low water column concentrations (<2.0 ppb) appears to have no adverse effects on the embryonic development of the studied species. There is evidence, however, that a high concentration (74 ppb) of TBT during fertilization can significantly reduce hatch success. The implication is that high interstitial concentrations of TBT (74 ppb), if chemically available, may inhibit the reproductive success of fishes that bury their eggs in contaminated sediment. Exposure of eggs during fertilization to 10-ppb TBT showed no significant reduction in hatching success.

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APPENDIX A

GRUNION LARVAE TEST

METHODS

A separate test was conducted in conjunction with the egg experiment to test the effects of TBT on newly hatched grunion larvae. During the hatching procedure, an additional subsample of eggs and sand was taken from the control group (those eggs fertilized and incubated in clean seawater) and hatched. Larvae (180) were distributed throughout the dosing system and held for 7 days. Three replicates, containing 10 individuals, were held in each of the 6 previously discussed dosing levels. Daily counts of live individuals were taken in each of the resulting 18 test containers.

Grunion larvae stay in a yolk sac stage from 7 to 10 days after hatching (May 1971). Active feeding generally begins 4 days after hatch and at 10 days posthatch capture success is generally greater than 70 percent. In this test the larvae were not fed, and the test was terminated as soon as the control group showed signs of progressing from the yolk sac stage (7 days). By not feeding the larvae, only endogenous feeding was examined, and the problems associated with "ration levels" were eliminated.

RESULTS

Although overall survival of yolk sac larvae was high throughout the 7-day test, results were similar to those of the egg test. Highest survival was obtained in the 0.73-ppb treatment, followed by the 1.65, 0.30, 0.04, and 0.12 treatments and the control, respectively. Mean percent survival throughout the 7-day test at the 0.73 ppb was 97 percent, contrasting with a lowest survival rate of 87 percent in the controls.

Survival in the controls and at the lowest dose level, 0.04 ppb, dropped drastically on the 7th day compared to the survival rate during the rest of the test. This drop in survival and examination of the remaining larvae prompted the decision to terminate the test as the unfed larvae appeared to be passing out of the yolk sac stage. On the 7th day, survival in the control dropped from 87 to 53 percent while survival remained high in the 0.73- and 1.65-dose levels at 93 and 90 percent, respectively.

DISCUSSION

A trend illustrated in this larval test, although not pronounced, follows that of the egg experiment and indicates a hormestic effect. The mechanisms of hormesis are not well understood and results found here pose some possibilities.

The high mortality on day 7 in the control and 0.04-ppb dose groups indicates the possibility of a faster development in these treatments. The low mortality rate throughout the test at the 0.73- and 1.65-ppb dose levels indicates a stimulatory effect of the toxicant at these levels. However, this stimulatory effect would only be limited to survival and not development, as these higher levels had not completely utilized the available yolk and did not appear to be experiencing starvation. Therefore, at these low levels TBT may create a less stressful environment for the larvae (i.e., limits bacterial or fungal growth, anesthetizes the larvae, etc.) while at the same time inhibiting growth and development.

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